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13. SUPPLEMENTARY NOTES

14. ABSTRACT
It is likely that the mild TBI and cognitive impairments observed among many of the troops returning from OIF and OEF result from repeated exposures to blast overpressure. Although the clinical symptoms of concussion are typically transient, there is both a cumulative risk for persistent damage due to repeated concussions, and a post-concussion period of greatest vulnerability to a second impact. Specific risk assessments and guidelines should be established for exposure to blast overpressure. We are using a preclinical model of blast overpressure in rats to investigate the cumulative effects of multiple blast exposures on neurologic status, neurobehavioral function, and brain histopathological endpoints. Repeated exposures to blast overpressure with varied inter-blast intervals are used to characterize and define the temporal window of brain vulnerability to repeated blast overpressure. We anticipate that these data will provide a critical first step in establishing rational risk guidelines and developing mitigation strategies.

15. SUBJECT TERMS
Traumatic Brain Injury (TBI), blast exposure, blast overpressure

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Table of Contents

INTRODUCTION:

It is likely that the mild TBI and cognitive impairments observed among many of the troops returning from OIF and OEF result from repeated exposures to blast overpressure. Although the clinical symptoms of concussion are typically transient, mild concussive brain injury can also result in persistent alterations in cognitive and emotional status. Based upon observations among athletes in contact sports, there is both a cumulative risk for persistent damage due to repeated concussions, and a post-concussion period of greatest vulnerability to a second impact, which may elicit subdural hematoma, vasospasm, brain swelling, elevated intracranial pressure, and occasionally death. Specific guidelines have been developed and periodically revised to establish when an athlete can resume their sport, based upon concussion severity and number. Similar risk assessments and guidelines should be established for exposure to blast overpressure. We are using a preclinical model of blast overpressure in rats to investigate the cumulative effects of multiple blast exposures on neurologic status, neurobehavioral function, and brain histopathological endpoints. Repeated exposures to blast overpressure with varied inter-blast intervals are used to characterize and define the temporal window of brain vulnerability to repeated blast overpressure. Spatial learning is assessed using the Morris water maze on days 8-12 post-BOP. Latencies to find the submerged platform are recorded along with swim patterns while doing so. Brains are then prepared for histopathological analysis to establish the extent of brain injury and to determine whether the brain injury severity increases with repeated exposure to blast, and diminishes with increased inter-BOP intervals. We anticipate that these data will provide a critical first step in establishing rational risk guidelines and developing mitigation strategies.

BODY:

Overview**:** A preclinical model of air blast injury in rats has been biomechanically validated and is being used to investigate the cumulative effects of repeated blast exposures on neurological status, neurobehavioral function, visual acuity, and brain histopathological endpoints. Varied inter-BOP intervals are used to identify the temporal window of brain vulnerability to repeated BOP. We anticipate that these data will provide a critical first step in establishing rational risk guidelines and developing mitigation strategies.

KEY RESEARCH ACCOMPLISHMENTS:

During this reporting period, we:

- Developed and utilized a new rat holder that records the static and dynamic pressures that each rat is exposed to in a non-rigid restraint device that neither shields the experimental subject nor contributes to the injury. This is a fundamental and necessary improvement over the previous holder.

- Using piezoresistive gauges to record both side-on and head-on pressures, mapped the blast simulation conditions along the longitudinal axis of the shock tube and established the optimal tube position to subject rats to repeated BOP exposures.
- Established Morris water maze, visual discrimination, EEG, and histopathological evaluation procedures that are now being effectively utilized in ongoing and soon-to-be completed experiments that will determine the extent to which re-exposure to a mild or moderate BOP 24 hrs following the first BOP exposure worsens outcome.
- Documented widespread fiber degeneration 3 days following exposure to BOP (86 kPa static peak pressure) that was absent in sham injured rats and was appreciably more prominent in those rats receiving a second airblast 24 hrs following the first.

During the initial reporting period, we recognized several significant technical impediments for our proposed research stemming from our rat holder and its restricted position at the mouth of the shock tube. Correction of these impediments was an essential prerequisite for progress on the defined milestones and collection of valid data. Blast generates an air shock front imparting effectively instantaneous increases in static and dynamic pressure conditions. The distinction regarding the incident blast flow conditions (i.e. static and dynamic pressures) and target loading have important implications with regard to the mechanisms for blast injury and cellular stresses as well as the proper experimental simulation of blast. In particular, the positioning of the experimental subject within the shock tube greatly influences exposure conditions and determines the relative contributions of side-on and dynamic pressures to the injury. After gaining a better appreciation of blast physics, we recognized that it is critical to be able to vary the position of rats within the shock tube using a holder that is minimally intrusive regarding rat exposure to shock wave and associated air movement. Our

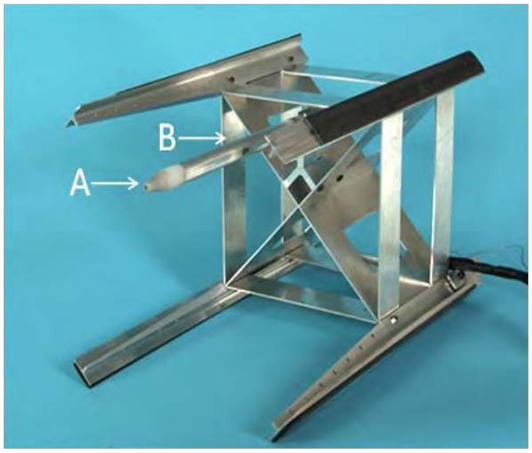


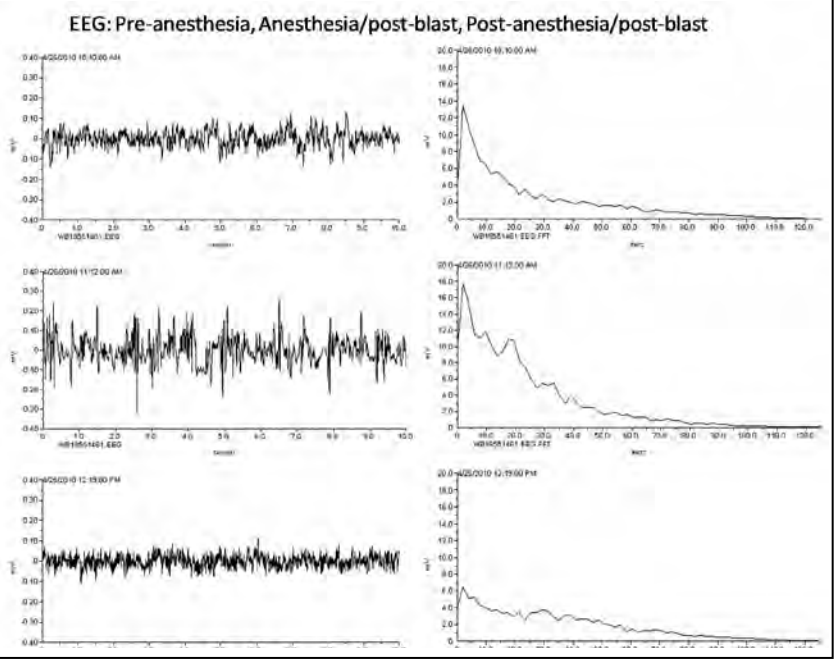
Fig 1. Rat holder with tip (A) and side (B) gauges

Table 1. Pressure readings at varied positions within the shock tube

Feet from Membrane	Peak Pressure (psi): Tip	Peak Pressure (psi): Side	Tip Impulse (psi-ms)	Side Impulse (psi-ms)
127 µ Thick Membrane				
5	13.07	11.27	140.40	126.39
9	12.80	10.73	135.18	119.99
12.5	14.00	11.47	118.28	92.39
15.5	14.40	10.20	60.00	10.33
254 µ Thick Membrane				
5	20.87	16.74	239.80	204.90
9	20.27	17.13	241.27	203.09
12.5	20.60	16.33	194.40	145.26
15.5	21.17	15.47	130.44	13.76

original rat holder, which fixed the rat at a set position at the mouth of the tube without gauges to record the specific pressure conditions at this position, was inadequate in this regard. We therefore designed a new rat holder that we now utilize that enables us to record the static and dynamic pressures that each rat is exposed to in a non-rigid restraint device that neither shields the experimental subject nor contributes to the injury (fig 1). The new holder can be positioned anywhere throughout the length of the shock tube and also better accommodates the instrumentation required for physiological recordings. As noted previously, although this unforeseen requirement has delayed our data collection from our original time projections, we are completely confident that we can complete the study within the overall time schedule with vastly improved, artefact-free data generated with a high fidelity simulation of blast.

Fig 2. Representative EEG tracings (l) and power spectra (r) after 16 psi BOP.



Recognizing that blast simulation conditions vary along the longitudinal axis of the shock tube, we have mapped these positional differences using comparative tip and side gauge recordings. As seen in table 1, greatest differences in pressure impulses become evident as one approaches the mouth of the shock tube, and are much more pronounced immediately outside the tube. In particular, the impulse ratio changed dramatically immediately outside the mouth of the tube, where total pressure impulse increased by factors of 100-fold reflecting an even more pronounced potential biomechanical influence of dynamic pressure (i.e. blast wind) in the exit-jet flow. This is a general concern with all exposures outside of a tube (such as occurred with our previous holder). The design of the new holder facilitates our control and quantitation of the individual components of blast contributing to injury. Through manipulation of controllable shock tube

Table 2. Neuropathology scores

	opt	CBW	SuG
Control	0	0	0
1 Blast	2	10	6
2 Blast	9	16	7

Scores (0, 1, or 2) were assigned based upon the level of silver impregnation in injured fibers (0-absent, 1–moderate, and 2-extensive). Left and right scores assigned by 2 blinded observers are summed (N=3 brains per treatment gp). Optic tract (opt), Cerebellar white matter (CBW), and Superficial grey layer of the superior colliculus (SuG).

experimental parameters (e.g. driver volume, tube position, Mylar membrane thickness, and type of gas), one can recreate a wide variety of conditions experienced in different blast scenarios, and also comparatively establish which of these parameters (e.g. peak pressure vs. impulse) has the greatest influence on physiological perturbations and injury mechanisms.

Characterizations and comparisons of the neurological and neurobehavioral outcomes in shams, single insult subjects, and rats re-exposed to a second BOP 24 hours after the first BOP are underway. In these experiments, we consistently position the rat 2.5 ft from the mouth of the shock tube. Visual discrimination and Morris water maze evaluations are in place and are being used to discern blast-induced disruptions in functional outcomes, along with EEG (Fig 2) and acute cardiovascular recordings. Although we do not yet have adequate subject populations to meaningfully statistically compare outcomes among the sham, single BOP, and repeated BOP treatment groups, with the required BOP conditions now defined and the assessment methodologies in operation, we anticipate completing this milestone in 2-3 months.

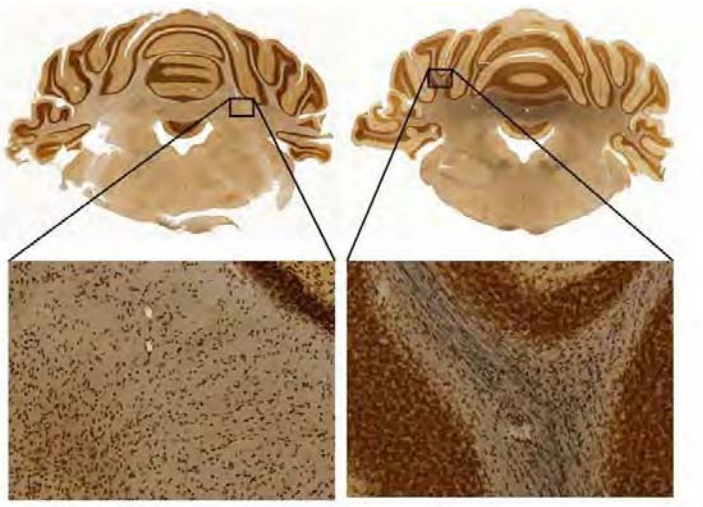
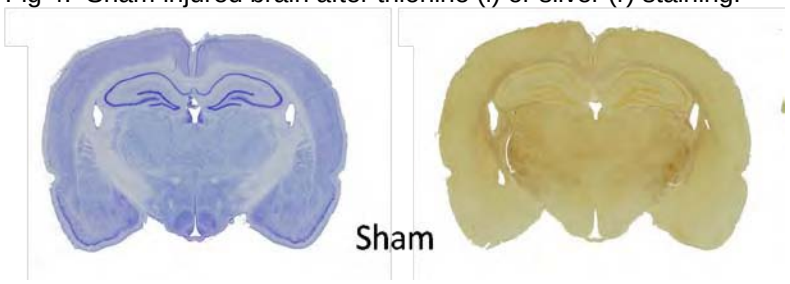


Fig 3. 10X (top) and 40X (bottom) photomicrographs of sham-injured (left) and blast – injured (right) rat cerebella. Axonopathy, which is absent in the sham-injured brain, is prominent as darkened, silver-impregnated fibers in the injured cerebellum on the right which was exposed to 20 psi airblast 2.5 ft from the mouth of the tube.

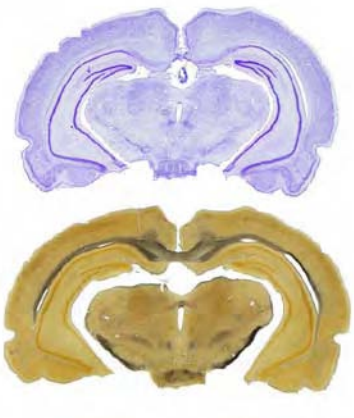
We have discerned BOP-induced neuropathological changes with these new exposure conditions and exacerbation of these changes by a second blast exposure 24 hrs following the first (Table 2). At approximately 86 kPa peak pressure intensities, brains of rats exposed to airblast typically are devoid of any obvious cell loss or injury, and instead most

Fig 4. Sham injured brain after thionine (l) or silver (r) staining.



typically show widespread fiber degeneration that is evident in silver-stained sections throughout all levels of the brain. Silver impregnation of fibers is widespread and is observed bilaterally, although not uniformly. The fiber degeneration is particularly prominent in the cerebellum (fig 3), optic tracts, and in commissural fibers of the corpus callosum. Axonopathy is also evident at higher magnification in small-caliber fibers as well. Brain injuries have been qualitatively similar in subjects exposed to airblast at other tube positions. In contrast, fiber degeneration is uniformly absent in brains of all the sham handled rats (Fig 4). Fiber degeneration is also clearly more pronounced in brains of rats receiving repeated airblast exposures than in those exposed to BOP only once (table 2). These neuropathological changes closely resemble those previously seen following 126 kPa airblast exposures at the mouth of the shock tube (fig 5). Similarly, LOC (reflected by recovery of righting and other reflexes) is more prolonged after the second BOP than is seen after one airblast exposure.

Fig. 5. Thionine- (top) and silver- (bottom) stained brain sections following exposure to 126 kPa airblast at the mouth of the tube. From Long et al., J. Neurotrauma 26:827-840, 2009.



REPORTABLE OUTCOMES:

Manuscripts

Long, J. B., Tong, L., Bauman, R. A, Atkins, J. A, Januszkiewicz, A. J., Riccio, C., Gharavi, R., Shoge, R., Parks, S., Ritzel, D. V., and Bentley, T. B. Blast-induced traumatic brain injury: using a shock tube to recreate a battlefield injury in the laboratory. IFBME Proceedings 30. 2010.

Abstracts/Presentations

Gharavi R.B., Shoge R.O., Long J.B., and Bentley T.B. Characterization of an air-driven shock tube used to recreate battlefield traumatic brain injuries. National Neurotrauma Symposium, June 2010.

Riccio C, Bentley T.B., and Long J.B. Blast overpressure injury in rats produces widespread fiber degeneration. National Neurotrauma Symposium, June 2010.

CONCLUSION:

Brain photomicrographs scored by blinded observers reveal modest BOP-induced brain injury that is exacerbated by exposure to a second 86 kPa BOP 24 hours later.

Neurobehavioral assessments of rats exposed to single and repeated 12 psi blasts are ongoing. We will soon complete the 1 day inter-BOP evaluations and will next evaluate rats exposed to BOP with 3 and 5 day inter-BOP intervals.

REFERENCES: NONE

APPENDICES: Attached

Blast-induced Traumatic Brain Injury: Using a Shock Tube to Recreate a Battlefield Injury in the Laboratory

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Abstract - **Explosive detonation has been a longstanding battlefield concern for the U.S. Army. Recently, emphasis has shifted to blast injury to the brain since blast has emerged as the predominant cause of neurotrauma in current military conflicts, and its etiology is largely undefined. Using a compression-driven shock tube to simulate blast, we are assessing the physiological, neuropathological, and neurobehavioral consequences of airblast exposure. Blast generates an air shock front imparting effectively instantaneous increases in static and dynamic pressure conditions. The positioning of the experimental subject within the shock tube greatly influences the exposure conditions and determines the relative contributions of these side-on and dynamic pressures to the injury. The pressure exposures and brain injuries resulting from airblast exposure at different shock tube positions are reviewed. Shock tube exposures provide survivable blast conditions under which striking neuropathological changes can be generated and TBI can be studied. These findings demonstrate that shock tube-generated airblast can cause TBI in rats, and point to the utility of this experimental tool in the development of effective therapies and countermeasures.**

Keywords – blast, traumatic brain injury, shock tube, shock wave, simulation, blast overpressure

I. Introduction

Blast has emerged as the predominant cause of casualties in Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF), with the majority of these injuries resulting from blast propagated by improvised explosive devices (IEDs). According to the Department of Defense's Defense Manpower Data Center [1], as of Feb 6, 2010, over 63% of US military casualties in OIF and OEF have been caused by explosive device weaponry. Among these casualties, the large majority of neurotrauma patients have closed head (i.e. nonpenetrating) injuries. Recent figures from the Department of Defense Military Health System [2] indicate that as of October 2009, there have been 152,939 medically-diagnosed non-penetrating traumatic brain injuries among service members in the U.S. military since FY2000, which is 98% of the overall TBI total. Other investigators, using post-deployment screening assessments such as the Post Deployment Health Assessment (PDHA) or Post Deployment Health Reassessment (PDHRA), have estimated even higher incidences of blast TBI (bTBI). For example, a recent RAND report estimates that 320,000 service members or 20% of the deployed force potentially suffer from TBI [3]. A similar summary from the Institute of Medicine estimates the prevalence of bTBI in deployed U.S. warfighters at 22% [4].

Despite claims to the contrary [5], it is widely recognized by leading authorities that bTBI can be produced at all severity levels, independent of a penetrating wound or being bodily thrown [4,6]. The etiology of primary blast-induced TBI is at this point largely undefined, although it appears that it may differ substantially from the penetrating brain injuries caused by ballistics or shrapnel in earlier conflicts. Body armor has made blast injuries survivable; consequently, to a large extent blast-induced nonpenetrating head injuries have emerged among troops who without body armor would have simply been killed in action as a result of injury to more vulnerable organs such as the lung [7]. Collectively, these statistics highlight the urgent need to advance medical care targeting the growing numbers of veterans with disabilities stemming from bTBI. This includes efforts to preclinically define the pathophysiological mechanisms underlying blast-induced TBI, to devise improved means to mitigate the risk of brain injury after blast exposure, and to identify rational therapeutic interventions. In this report, we describe considerations that go into the preclinical modeling of blast TBI in the laboratory and our experimental efforts to simulate blast TBI using a compressed air-driven shock tube.

An explosion is caused by the release of energy resulting from the nearly instantaneous chemical conversion of a solid or liquid into a gas [5-10]. The gaseous detonation products expand rapidly from their point of origin and compress the surrounding medium (usually air or water), generating a blast wave. Mechanical and thermal energy are transferred into the surrounding air or water as well as into objects or bodies within proximity to the blast. In air, the most distinctive feature of the blast wave is the supersonic shock front, which is the leading element of the pressure disturbance through which there is a nearly instantaneous change in all gas-dynamic conditions of the air (pressure, density, flow velocity, and temperature). For a typical explosion in a free field, the pressure-time relationship has been described by Friedlander as an initial rapid rise in pressure, followed by an exponential-like decay which may rebound below the ambient pressure and produce a negative pressure phase, thereafter rising back to baseline [6,10].

The shock front is associated with a blast wind (dynamic

pressure) resulting from the kinetic energy imparted to the air as it is traversed by the shock wave [6-10]. Thus, following an explosion, an individual exposed to a blast wave will be exposed to a step increase in static pressure as well as a high velocity wind [6-10]. With time and distance, the peak pressure and velocity of the blast wave weaken. Near the source of the explosion the overpressure decreases approximately with the inverse cube of the distance from the origin, but at greater distances it decays inversely with distance as an acoustic wave [10]. The distinction regarding the incident blast flow conditions (i.e. static and dynamic pressures) and target loading have important implications with regard to the mechanisms for blast injury, imparted loading, and cellular stresses as well as the proper simulation of blast in the laboratory [8].

Blast waves are often described solely by their peak side-on (or static) overpressure, which is an incomplete characterization that does not represent the target loading pressure. Although the static pressure profile is an important component of blast insult, it is by no means the only relevant energy component, particularly for victims within the area of the fireball, where kinetic energy of the flow dominates [8].

In a shock tube, the side-on pressure measurement commonly used to characterize peak overpressure is the pressure detected on a surface aligned parallel to the blast wave propagation, which does not offer aerodynamic resistance and therefore does not experience the kinetic energy component of the flow. The reflected pressure experienced by a target obstructing the flow may be many-fold higher than the unobstructed static pressure component [8]. In terms of physiological significance to blast TBI, the critical biomechanical loading to the experimental subject is determined from both the static (Ps) and dynamic pressure (Pd) of the blast wave and the geometry of the structure [8,9]. The three static overpressure parameters of greatest importance are the peak value of P (i.e. peak overpressure), its duration, and the impulse (i.e. area under the pressure-time curve); rise time and decay rate are also important for visco-elastic biologic materials. When simulating blast in a shock tube, the relative contributions of Ps and Pd to the imparted loading vary with the position along the long axis of the tube, which can be altered to simulate particular blast exposure conditions. To clearly monitor the potential contributions of both these parameters to experimental bTBI in a subject exposed to airblast at different tube positions, we have devised a rat holder that incorporates piezoresistive gauges to record both side-on and head-on pressures. Preliminary findings with this device are described below along with associated histopathological descriptions of the brain injuries resulting from single or repeated air blast exposures.

II. Materials and Methods

Sprague Dawley rats (275-325 g) were anesthetized with isoflurane and subjected to survivable blast overpressures that were generated using an air-driven shock tube with Mylar membranes rupturing at predetermined pressured thresholds. The shock tube (Fig. 1) consists of a 2.5 ft long compression chamber that is separated from a 15 ft long

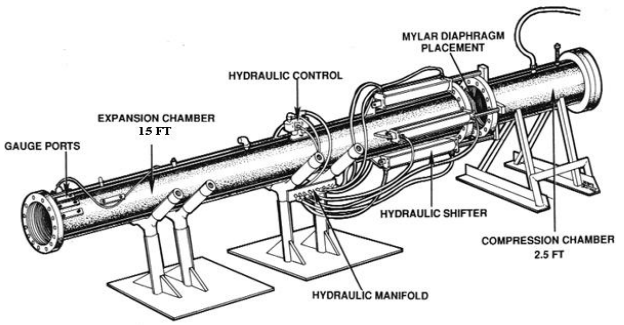

Fig. 1 Shock tube

expansion chamber by polyester Mylar membranes (DuPont, Wilmington, DE) of different thicknesses. Both chambers are 1 ft in diameter. Using an air compressor, the compression chamber is pressurized with room air, causing the Mylar membrane to rupture at a pressure that is linearly dependent upon the thickness of the Mylar sheet(s) separating the two chambers. Rats were placed in a transverse prone position in a holder secured 2.5 ft within the mouth of the tube. Piezoresistive gauges (Endevco) incorporated into a low profile aluminum holder (Fig. 2) were used to record the static (i.e. side-on pressure) and dynamic pressure (i.e. blast wind) at multiple positions, including the tube positions where rats were exposed. After airblast or sham handling, rats were quickly removed from the tube and returned to their home cages. In some cases, rats were reexposed to airblast 24 hours after the initial exposure. Three days after their final blast exposure or sham handling, rats were anesthetized and following perfusion fixation with a 4% paraformaldehyde solution, brains were sectioned (30 um) and silver impregnated to study fiber degeneration (i.e. axonopathy). Sections were independently scored by 2 blinded observers. Scores (0, 1, or 2) were assigned to specific brain regions based upon the extent of silver impregnation in these neuroanatomical structures.

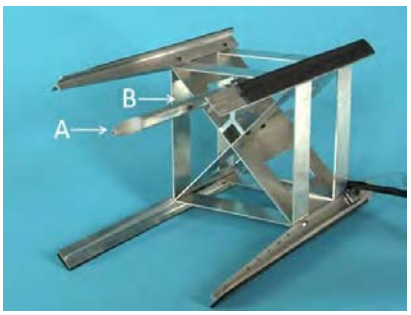

Fig 2. Rat holder with tip (A) and side (B) gauges

III. Results

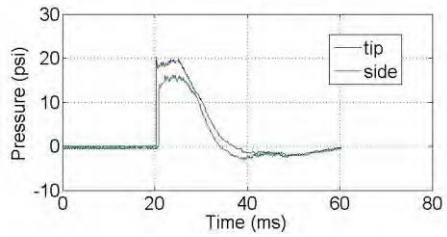

Fig 3. Blast pressure recordings when positioned 12.5 ft from a 1000 µ Mylar membrane. Tip gauge recording in blue, side gauge in green.

Fig. 3 shows typical pressure tracings recorded with gauges placed 2.5 ft from the mouth of the tube (i.e. 12.5 ft from the Mylar membrane). The markedly higher peak pressure and impulse recorded from the tip gauge measuring the ‚total pressure' reveal that Ps only partly accounts for load conditions experienced at this point. As anticipated, Ps and Pd varied somewhat along the axis of the tube (Table 1). In particular, although peak pressures were relatively consistent across locations, pressure impulses measured from both gauges decreased substantially closer to the mouth of the tube and impulse ratios increased (Fig. 4), revealing a greater relative contribution of Pd to biomechanical loading at locations closer to the open end of the tube. The impulse ratio changed dramatically immediately outside the mouth of the tube, where total pressure impulse increased by factors of 100-fold reflecting an even more pronounced potential biomechanical influence of Pd in the exit-jet flow. The exit-jet flow also includes a quasi-steady "Mach disc" shock which greatly affects specimen response. Positional variations were consistent across all Mylar membrane thicknesses and associated pressures.

Table 1. Pressure Readings for Different Membrane Thicknesses at Varying Positions within the Blast Tube

Feet from Membrane	Peak Pressure (psi): Tip	Peak Pressure (psi): Side	Tip Impulse (psi-ms)	Side Impulse (psi-ms)
500 µ Thick Membrane				
5	13.07	11.27	140.40	126.39
9	12.80	10.73	135.18	119.99
12.5	14.00	11.47	118.28	92.39
15.5	14.40	10.20	60.00	10.33
1000 µ Thick Membrane				
5	20.87	16.74	239.80	204.90
9	20.27	17.13	241.27	203.09
12.5	20.60	16.33	194.40	145.26
15.5	21.17	15.47	130.44	13.76

Values are averaged from 3 recordings

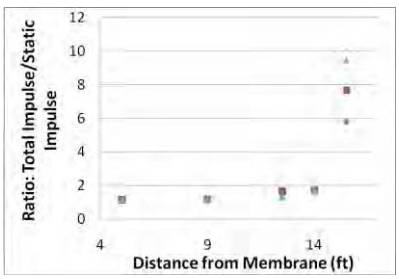

Fig 4. Impulse ratios calculated from the average of 3 tip gauge and side gauge recordings at varied tube positions and Mylar membrane thicknesses. Square – 500 µ thickness, Triangle – 750 µ thickness, Diamond – 1000 µ thickness, and Cross – 1400 µ thickness.

As previously described [12], at approximately 20 psi peak pressure intensities, brains of rats exposed to airblast typically were devoid of any obvious cell loss or injury, and instead most typically showed widespread fiber degeneration that was evident in silver-stained sections throughout all levels of the brain (Fig. 4). The neuropathological changes produced 2.5 ft from the mouth of the tube (12.5 ft from the Mylar membrane) were widespread and were observed bilaterally, although not uniformly. The fiber degeneration was particularly prominent in the cerebellum, optic tracts, and in commissural fibers of the corpus callosum. Axonopathy was evident at higher magnification in small-caliber fibers as well. Brain injuries were qualitatively similar in subjects exposed to airblast at other tube positions [12; unpublished observations]. In contrast, fiber degeneration was absent in brains of all the sham handled rats (Fig. 5). Fiber degeneration was also clearly more pronounced in brains of rats receiving repeated airblast exposures than in those exposed only once (Table 2).

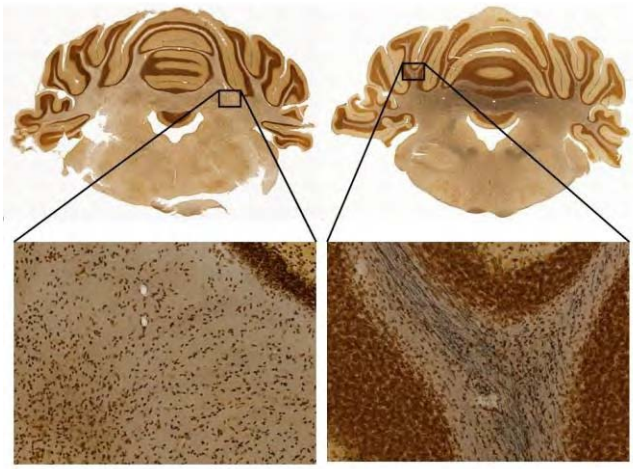

Fig 5. 10 X (top) and 40X (bottom) photomicrographs of sham-injured (left) and blast –injured (right) rat cerebella. Axonopathy, which is absent in the sham-injured brain, is prominent as darkened, silver-impregnated fibers in the injured cerebellum on the right which was exposed to 20 psi airblast 2.5 ft from the mouth of the tube.

Table 2. Fiber degeneration neuro-pathology scores

	opt	CBW	SuG
Control	0	0	0
1 Blast	2	10	6
2 Blast	9	16	7

Scores (0,1, or 2) were assigned based upon the level of silver impregnation in injured fibers (0-absent, 1–moderate, and 2-extensive). Left and right scores assigned by 2 blinded observers are summed (N=3 brains per treatment gp). Optic tract (opt), Cerebellar white matter (CBW), and Superficial grey layer of the superior colliculus (SuG).

IV. Discussion

These data confirm that blast simulation conditions vary along the longitudinal axis of the shock tube and represent a first step at mapping these positional differences. In general, the comparative tip and side gauge recordings

reveal greatest differences in pressure impulses which become evident as one approaches the mouth of the shock tube, and are much more pronounced immediately outside the tube. The relative influences of tube position on these recordings are consistent across Mylar membrane thicknesses and associated driver pressures.

As presently configured, the side-on pressures of blastwaves generated in this shock tube are of an approximately 12-16 msec duration. Since explosive blasts in the field typically yield shorter blastwaves (typically ranging from 2 to 10 msec), it will be desirable to modify the tube to better simulate this relevant blast waveform. This can be accomplished by altering the ratio of the dimensions of the driver (i.e. compression chamber) relative to the expansion chamber. Through manipulation of these experimental parameters (e.g. driver volume, tube position, Mylar membrane thickness, and type of gas), one can recreate a wide variety of conditions experienced in different blast scenarios, and also comparatively establish which of these parameters (e.g. peak pressure vs. impulse) has the greatest influence on physiological perturbations and injury mechanisms.

Rats exposed to approximately 20 psi airblast displayed neuropathological changes that were qualitatively quite similar to those observed in rats and pigs after different exposure conditions [11-13]. In all cases, injury was most prominent as widespread fiber degeneration that was not associated with cell loss or injury. Although the numbers of subjects in each treatment group are small, it is noteworthy that rats receiving two airblast exposures had noticeably greater neuropathological changes than either the single- or sham-exposure groups.

The widespread use of explosive weaponry (e.g. IEDs) in OIF and OEF has prompted a surge in biomedical research to address the consequences of blast exposure, the relevant injury mechanisms, and potential countermeasures. Shock tubes, which have been used for decades in blast biophysics research, are now increasingly employed as a research tool for biomedical research as well. As the use of shock tubes and other experimental models of blast expand, it is critical that they be used in a manner that most effectively simulates explosive blast conditions, recognizing that creation of an injury does not constitute validation of an injury model. For example, immediately outside the mouth of a shock tube conditions are very complex with high flow gradients such that slight changes in position impart large changes in pressure conditions. Practically all flow energy is converted to a collimated jet at the shock tube exit yielding extreme dynamic pressure and negligible static pressure as end wave rarefaction abruptly reduces static pressure and greatly accelerates flow. As a consequence, despite the appearance of injuries and pathophysiological responses, experimental subjects placed outside the mouth of the shock tube in an attempt to model blast exposure are in all likelihood experiencing extremely different loading and injury phenomena than those resulting from explosive blast, and might also yield disparate neuropathological changes [8,11, 14,15]. It is therefore essential that exposure conditions be carefully monitored and considered to validate the fidelity of the experimental model. The measurements of the physical conditions and associated neuropathological outcomes described in this report represent a step toward that objective.

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References

1. DMDC at http://siadapp.dmdc.osd.mil/personnel/CASUALTY/castop.htm.
2. MHS at (http://www.health.mil/Pages/Page.aspx?ID=49)
3. Tanielian T, Jaycox, LH, eds. (2008) Invisible Wounds of War: Psychological and Cognitive Injuries, Their Consequences, and Services to Assist Recovery. Santa Monica, CA: RAND Corporation
4. Institute of Medicine (2009) Gulf War and Health, Volume 7: Long-Term Consequences of Traumatic Brain Injury. Washington, DC: The National Academies Press
5. Champion HR, Holcomb JB, Young LA (2009) Injuries from explosions: physics, biophysics, pathology, and required research focus. J Trauma 66:1468–77, discussion 77
6. Ling G, Bandak F, Armonda R et al. (2009) Explosive blast neurotrauma. J Neurotrauma 26:815–25
7. Wightman JM, and Gladish SL (2001) Explosions and blast injuries. Ann. Emerg. Med. 37:664–678.
8. Benzinger TL, Brody D, Cardin S et al. (2009) Blast-related brain injury: imaging for clinical and research applications: report of the 2008 St. Louis workshop. J Neurotrauma 26:2127-2144
9. Cernak I, Noble-Haeusslein LJ (2010) Traumatic brain injury: an overview of pathobiology with emphasis on military populations. J Cereb Blood Flow Metab. 30:255-66.
10. Leung LY, VandeVord PJ, Dal Cengio AL et al. (2008) Blast related neurotrauma: a review of cellular injury. Mol Cell Biomech 5:155–68
11. Long JB, Bentley TL, Wessner KA et al. (2009) Blast overpressure in rats: recreating a battlefield injury in the laboratory. J Neurotrauma 26:827–40.
12. Bauman RA, Ling G, Long L et al. (2009) An introductory characterization of a combat-casualty-care relevant swine model of closed head injury resulting from exposure to explosive blast. J Neurotrauma 26:841-860.
13. Garman R, Jenkins LW, Bauman RA et al. (2009) Blast overpressure injury in rats with body protection produces acute and subacute axonal, dendritic and synaptic neuropathology. J Neurotrauma 26:A-53.
14. Jaffin JH, McKinney L, Kinney RC et al. (1987) A laboratory model for studying overpressure blast injury. J Trauma 27:349-356.
15. Svetlov SI, Prima V, Kirk DR et al. (2010) Morphologic and biochemical characterization of brain injury in a model of controlled blast overpressure exposure. J Trauma in press.

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Characterization of an Air-Driven Shock Tube used to Recreate Battlefield Traumatic Brain Injuries

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Blast induced traumatic brain injury (bTBI) has emerged as the predominant neurotrauma in the current military conflicts and the global war on terrorism. Estimates for the number of bTBI casualties range from 150,000 to 320,000 or 20% of deployed U.S. troops. Unfortunately, bTBI treatment is hindered by incomplete knowledge of the physical and biological mechanisms involved. To increase understanding of such etiologies and for development of new therapies, we are creating a lab-based, small animal model of bTBI using an air-driven shock tube. Characterization of the injury inducing forces produced by the shock tube are necessary to ensure the fidelity and relevance of the overpressure model to the forces experienced by personnel exposed to explosive blasts in the field. Experimentally generated blast overpressures have largely been characterized by their static, or side-on, pressure while not taking into account the total, front-on, pressure which includes a dynamic pressure component in the form of a blast wind. The objective of this project is to characterize the incident flow conditions generated by an air-driven shock tube through the measurement of static and total pressures. The shock tube consists of a 4.6m long X 0.3m diameter expansion tube and a 0.9m X 0.3m diameter compression chamber, separated by a mylar membrane. The mechanical build up of air pressure in the compression chamber causes the membrane to rupture, yielding a blast wave. Piezoresistive gauges recorded static and total pressures just outside the tube and 0.3, 0.8, 1.8 and 3.0 meters from the tube mouth. Three overpressure parameters were calculated from the static and total pressure vs time data: peak static and total pressure, blast wave duration, and the integral of pressure over time (impulse).

Peak total pressures of 145kPa with durations of 12-16 ms were generated using 356µm membranes. Within the tube, static and total peak pressures were fairly constant, though wave durations and impulses decreased towards the tube mouth. Peak total pressure was 20% higher than peak static pressure at all distances from the blast origin. Directly outside the tube the ratio of total to static impulse increased 100 fold, indicating a substantial contribution of dynamic pressure within the expanded volume of this location. Although loading conditions differed, rats positioned .3 m inside and immediately outside the tube had similar brain injuries, which typically included widespread fiber degeneration. The results contribute to mapping pressure components along a shock tube axis and illustrate static pressure partly accounts for the loading conditions resulting from airblast exposure. Blast tube conditions can be manipulated to achieve pressure profiles that accurately simulate free field blasts in order to better understand blast induced overpressure's role in initiating neuropathological mechanisms. (Supported by CDMRP Award W81XWH-08-2-0017)

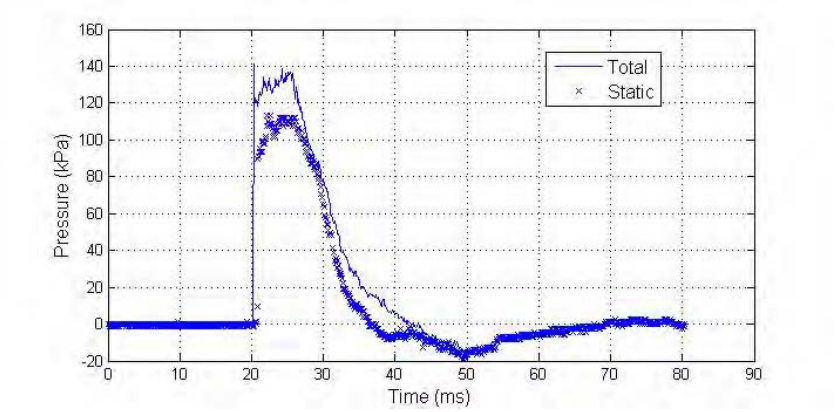

Figure 1. Total and static pressure waveform generated inside the shock tube using 356 micron thick mylar membrane .3 meters from the mouth of the shock tube. Here the total pressure is larger than the static pressure due to the dynamic pressure component and has a longer duration.

Total and Static Pressure Readings at Different Distances from Shock Tube Mouth Values obtained using 356 micron thick Mylar Membrane				
Distance within Tube (m)	Avg. Peak Total Pressure (kpa)	Avg. Peak Static Pressure (kpa)	Total Impulse (kpa-ms)	Static Impulse (kpa-ms)
Compression Chamber	345.76			
3.0	175.82	113.53	1998.05	1612.91
1.8	142.28	114.45	1864.77	1555.32
0.8	142.26	113.53	1450.15	1037.04
0.3	146.87	111.24	1281.23	542.55
Outside of tube	139.83	100.39	990.93	97.99

BLAST OVERPRESSURE INJURY IN RATS PRODUCES WIDESPREAD FIBER DEGENERATION

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Blast-induced traumatic brain injury (bTBI) has emerged as the leading cause of neurotrauma in current military operations. Troops exposed to blast who do not require immediate medical attention may still be affected by bTBI; the number of veterans with disabilities caused by bTBI is growing, and, at present, the etiology of bTBI is not well defined. Animal models must be used to increase understanding of the pathophysiological mechanisms of bTBI and develop new preventative and therapeutic approaches to bTBI. In addition to closely reproducing the loading conditions and biomechanical features associated with explosive blast, the model(s) must reliably produce bTBI analogous to the injuries seen in blast-exposed casualties. We have developed such a model with graded simulated blast exposures created with a compressed air-driven shock tube. The neuropathological consequences are presented here. Male S-D rats (350-450 g), anesthetized with a time limited exposure to 5% isoflurane, were placed in a transverse position within the blast tube and exposed to a survivable blast pressures (86 kPa static peak pressure) either once or twice separated by 24 hrs. The static and dynamic pressures produced by the air blasts in the tube were recorded by side-on and tip gauges. Three days after the final blast exposure or sham handling, rats were anesthetized and following perfusion-fixation, coronal sections were prepared (30 μm) for staining using silver, Nissl, and Fluoro Jade procedures. Sections from 11 defined brain regions were independently read by 2 blinded observers and assigned scores (0, 1, 2) based upon the neuropathological features observed. Although brains of rats exposed to either one or two 86 kPa airblasts were typically devoid of any obvious cell loss or injury when assessed using either Nissl or Fluoro Jade stains, they consistently showed widespread fiber degeneration that was conspicuous in the silver-stained sections throughout all levels of the brain. Fiber degeneration unaccompanied by cell loss or injury is consistent with previous reports of bTBI (Long et al., J Neurotrauma 2009; Garman et al., J Neurotrauma 2009). Fiber degeneration was particularly pronounced in the cerebellum, optic tracts, and in the commissural fibers of the corpus callosum. Fiber degeneration was not seen in brains of sham handled rats and was appreciably more prominent in those rats receiving repeated airblast exposure than in those exposed only once. These worsened outcomes indicate a possible cumulative effect of TBI or an exacerbation of injury due to prior blast exposure. These findings reinforce indications from previous studies of the prominence of axonal injury as the principal neuropathological hallmark of bTBI. Moreover, these results illustrate the utility of the shock tube as an experimental tool to reproducibly simulate with high fidelity blast exposure conditions and establish the etiology of bTBI.